DESIGN A SETUP FOR PREPARATION OF CHITOSAN MICROBEADS BASED ON ECONOMIC AND SIZE CONSIDERATION

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Bachelor of Technology in

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Submitted

by

Ashutosh Samal

Under the Guidance of

Prof. Devendra Verma

D**epartment of Biotechnology & Medical Engineering**

National Institute of Technology

Rourkela-769008

National Institute of Technology

Rourkela

CERTIFICATE

This is to certify that the thesis entitled

"DESIGN A SETUP FOR PREPARATION OF CHITOSAN MICROBEADS BASED ON ECONOMIC AND SIZE CONSIDERATION"

Submitted by Mr. Ashutosh Samal in partial fulfilment of the requirements for the award of Bachelor of Technology Degree in Biomedical Engineering at National Institute of Technology, Rourkela is an authentic work carried out by him under my guidance.

To the best of my knowledge the matter embodied in the thesis has not been submitted to any University/Institute for the award of any Degree or Diploma.

Dr. Devendra Verma **Dated: 11 May 2015**

Department of Biotechnology and Medical Engineering.

National Institute of Technology Rourkela-769008

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Ashutosh Samal

Roll no 111BM0529

Dept. of Biotechnology & Medical Engineering

ABSTRACT

A setup was designed to prepare chitosan microbeads under optimum conditions. Chitosan microbeads find their wide range of applications in drug delivery systems, immobilisation of cells, enzymes and antibodies, as chromatographic supports and adsorbents for removal of metal ions from organic and aqueous solutions. Conventional methods of preparation of chitosan microbeads include spray drying, emulsification. In the present study the chitosan gel microbeads fabrication is done by extruding chitosan solutions through a syringe needle under centrifugal force. Chitosan drops were released from the needle at a definite rate depending upon the rotational speed of the centrifuge. These drops interact with 10% TPP solution, which act as a cross linker, to form microbeads. Microbeads of uniform diameter can be obtained by optimising the pH of TPP solution to 4 using acetic acid. The microbeads formation of varying diameters was observed by varying the chitosan solution concentration in the range of 1%-2%.

Keyword: microbeads, centrifugal force, cross linker

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Chapter 1

INTRODUCTION

INTRODUCTION

Chitosan:-

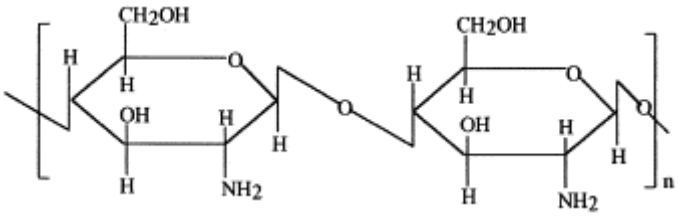


Fig 1.1: Structure of chitosan

Chitosan is a direct polysaccharide made out of arbitrarily distributed d-glucosamine and N-acetyl-d-glucosamine units linked by β-(1-4) glycosidic bonds. It can be obtained by alkaline deacetylation of chitin removed from the shells of shrimps, crabs, and lobster. Among all the properties that chitosan has ability to form films, polyoxysalt formation and chelate metal ions. Free amine as well as hydroxyl groups present in chitosan gives rise to different chitosan derivatives.

Why chitosan is preferred:-

chitosan is utilized as a filler as a part of tablets; as a bearer in controlled-discharge medications; to enhance the way certain medications break down; and also to coat some drug that has bitter tastes taken in mouth. Chitosan can be applied directly to places to help donor tissue rebuild itself.

Chitin derivatives and their proposed uses

Derivative	Examples	Potential uses
N-Acyl chitosans	Formyl, acetyl, propionyl, butyryl, hexanoyl,	Textiles, membranes
	octanoyl, decanoyl, dodecanoyl, tetradecanoyl,	and medical aids
	lauroyl, myristoyl, palmitoyl, stearoyl, benzoyl,	
	monochloroacetoyl, dichloroacetyl, trifluoroacetyl,	
	carbamoyl, succinyl, acetoxybenzoyl	
N-Carboxyalkyl	*N*-Carboxybenzyl, glycine-glucan (*N*-carboxy-	Chromatographic
(aryl) chitosans	methyl chitosan), alanine glucan, phenylalanine	media and metal
	glucan, tyrosine glucan, serine glucan, glutamic	ion collection
	acid glucan, methionine glucan, leucine glucan	
N-Carboxyacyl	From anhydrides such as maleic, itaconic, acetyl-	?
chitosans	thiosuccinic, glutaric, cyclohexane 1,2-dicarbox-	
	ylic, phthalic, *cis*-tetrahydrophthalic, 5-norbo-	
	rnene-2,3-dicarboxylic, diphenic, salicylic, tri-	
	mellitic, pyromellitic anhydride	
o-Carboxyalkyl	*o*-Carboxymethyl, crosslinked *o*-carboxymethyl	Molecular sieves,
chitosans		viscosity builders,
		and metal ion collec-
		tion

Fig 1.2: Types of chitosan and its uses

Advantages of Chitosan bead Formation:-

Chitosan offers awesome points of interest for ionic interactions due to its cationic nature. Biocompatible cross-linked chitosan microbeads can be produced by the interaction of chitosan with TPP leads, which can be productively utilized in protein and vaccine delivery. The hydrophilicity, cross-linking can allow passage of drug release and augment its scope of potential applications in drug delivery such as transport of insulin.

Sodium tripolyphosphate :

Sodium salt of the polyphosphate penta anion is an inorganic compound with molecular formula $Na_5P_3O_{10}$.

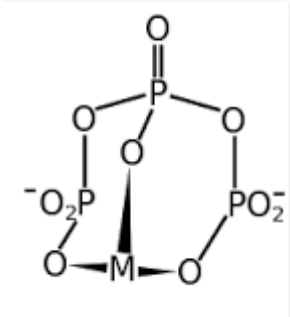


Fig 1.3: TPP structure

Glacial acetic acid:

Water free(anhydrous) acetic acid is also called glacial acetic acid..

Fig 1.4: Acetic acid structure

Chapter 2

REVIEW OF LITERATURE

2. Review of Literature:

Microbeads made of chitosan has a wide range of applications in the food industry, agriculture, biomedicine and the pharmaceutical and cosmetic industry owing to their low toxicity, biocompatibility, biodegradability, high binding capacity to specific chemical species, and the ability to adsorb or release molecules in response to external signals or stimuli.

Chitosan microbeads can be formed by different conventional methods such as: spray drying, atomisation, micromolding, photolithography

Dried particulate can be formed by spraying the fluidic stated sample to hot drying medium. It is a process of continuous drying. In this process concentrated feedstock is continuously given to the drying chamber. The heater will help to remove the extra water content. At the final stage of separation electrostatic precipitators, bag filters, cyclones may be used [5-7].

Chitosan microspheres of a small particle size and with good sphericity were obtained using spray-drying method on treating it with a cross-linking agent [10].

Many microengineering techniques have been developed for the production of microbeads, such as photolithography, micromolding, microfluidic routes, membrane emulsification and microchannel emulsification. The main advantage of using these methods is the ability to accurately control the bead size, shape, uniformity and morphology [8].

Micro-gel beads (200–1,200 μm in diameter) were produced by atomization of sodium alginate solution or a sodium alginate-perfluorocarbon mixture with a rotating disk [9].

Alginate microspheres reinforced with chitosan were prepared using emulsification/ionotropic gelation. Strengthened chitosan-covered microspheres were obtained by a continuous system, so as to improve the covering methodology, minimize protein loss. Round uncoated microspheres with a mean width of 20 μm and exemplification effectiveness over 89% were obtained using this technique [11]

In the presence of tripolyphosphate chitosan was made to react with sodium alginate for bead formation. Spherical beads were produced with diameter in the range 0.78–0.92 mm and 13–90% encapsulation efficiency [12]

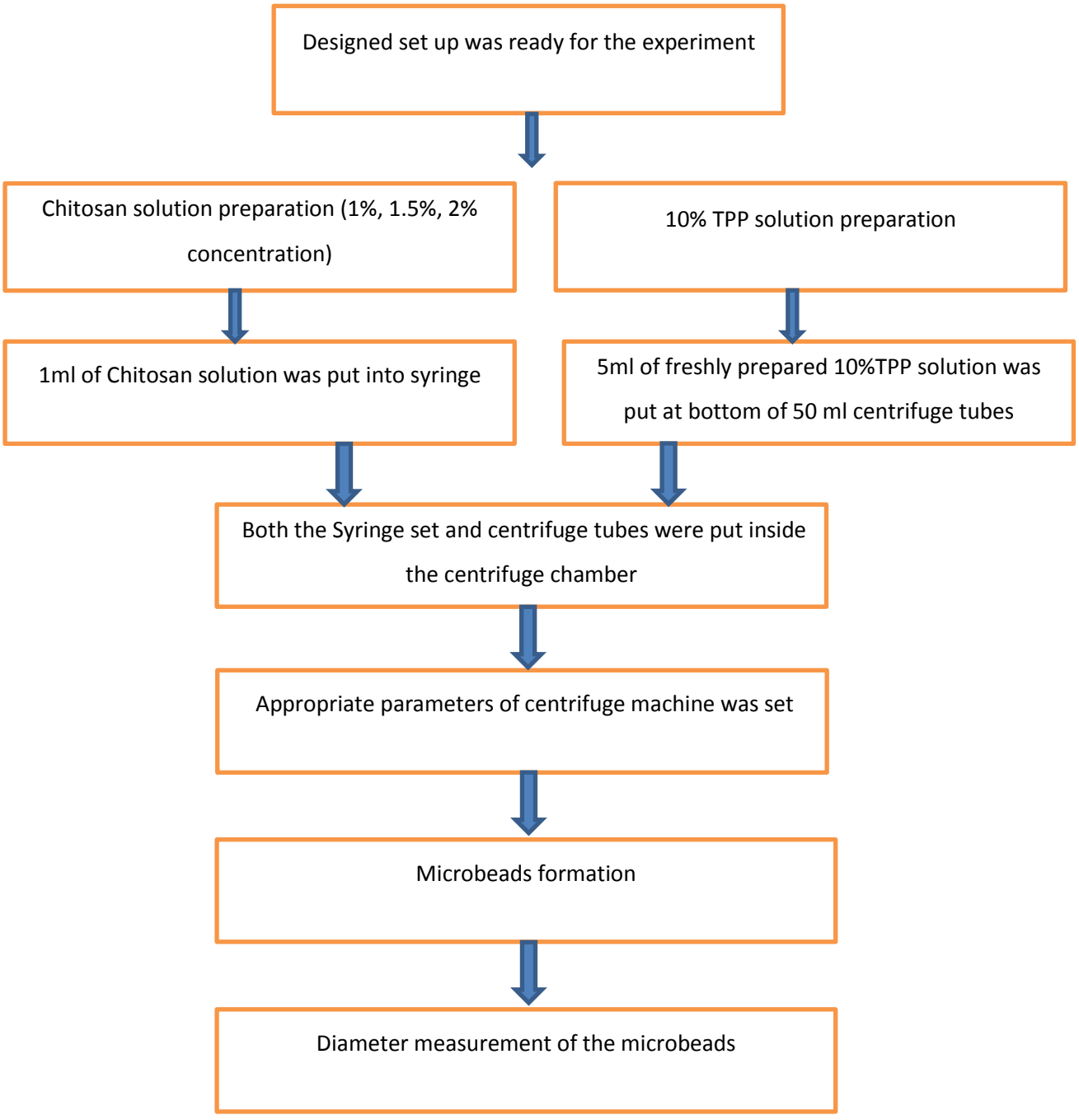
Using extrusion and spheronization technology chitosan microbeads could also be successfully prepared using the combination of Avicel RC-591 and chitosan [13]

Chapter 3

EXPERIMENTAL

3. EXPERIMENTAL

3.1 OVERVIEW



3.2 MATERIALS AND METHODS

3.2.1 Set up design:-

Fig 3.2.1: 2ml syringe with holes on both sides

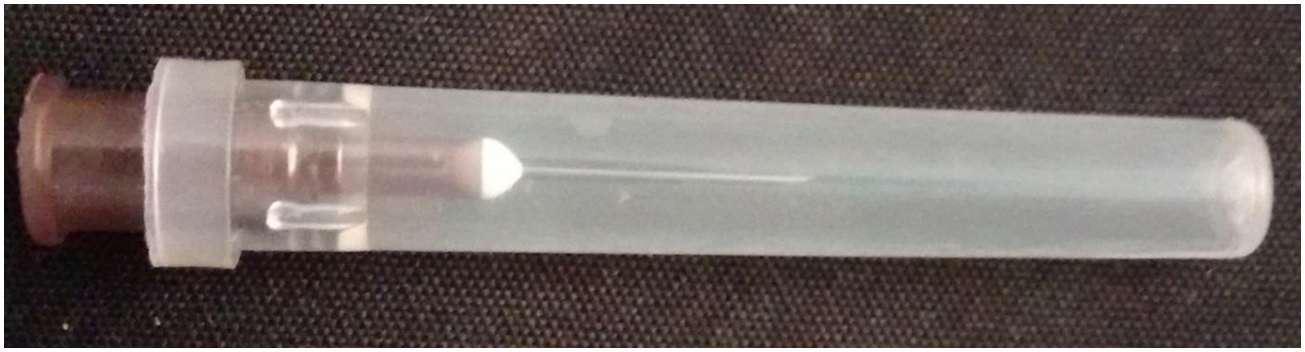


Fig 3.2.2: Needle

Fig 3.2.3: Needle packet

Needle	Nominal Outer Diameter			Nominal Inner Diameter			Nominal Wall Thickness		
Gauge	*inches*	*mm*	*tol. inches (mm)*	*inches*	*mm*	*tol. inches (mm)*	*inches*	*mm*	*tol. inches (mm)*
26s	0.01865	0.4737	±0.00025 (±0.0064)	0.005	0.127	±0.00075 (±0.019)	0.0068	0.1734	±0.00025 (±0.0064)

Fig 3.2.4: Needle specification

Fig 3.2.5: Plunger and plunger tip

Fig 3.2.6: Top view of the final setup

Fig 3.2.7: The final setup

- A hole was made at the centre of the cap of the centrifuge tube using drilling machine.
- The diameter of that hole should be equal to the diameter of the 2ml syringe to be inserted.
- Another two holes were made on both side of the centrifuge tube and 2ml syringe so as to facilitate the flow of air inside which forces the chitosan solution to come out from the needle.
- The cap along with the syringe tube was then fitted tightly onto the centrifuge tube.
- The 2ml syringe contains chitosan solution whereas the base of centrifuge tube contains 10% TPP solution.

3.2.2 Chitosan solution preparation:-

- Chitosan solution of different concentrations were prepared as below:-
  - ✓ 1% solution: - In a beaker 0.75ml of acetic acid was taken and 0.25gm of chitosan was added to it. Water was added to the above solution mixture to level it up to 25 ml.
  - ✓ 1.5% solution: - In a beaker 1ml of acetic acid was taken and 0.375gm of chitosan was added to it. Water was added to the above solution mixture to level it up to 25 ml.
  - ✓ 2% solution: - In a beaker 1.5ml of acetic acid was taken and 0.5gm of chitosan was added to it. Water was added to the above solution mixture to level it up to 25 ml.
  - ✓ 2.5% solution: - In a beaker 2ml of acetic acid was taken and 0.625gm of chitosan was added to it. Water was added to the above solution mixture to level it up to 25 ml.

- These contents were put in the beaker with magnetic bead. And the beaker was put on the magnetic stirrer.
- The rotating magnetic field cause to stir the magnetic bead immersed in the solution to spin very quickly, thus stirring it.
- The parameters of the magnetic stirrer were maintained: - Its RPM was set to 220 and the operational temperature was set to 55C.

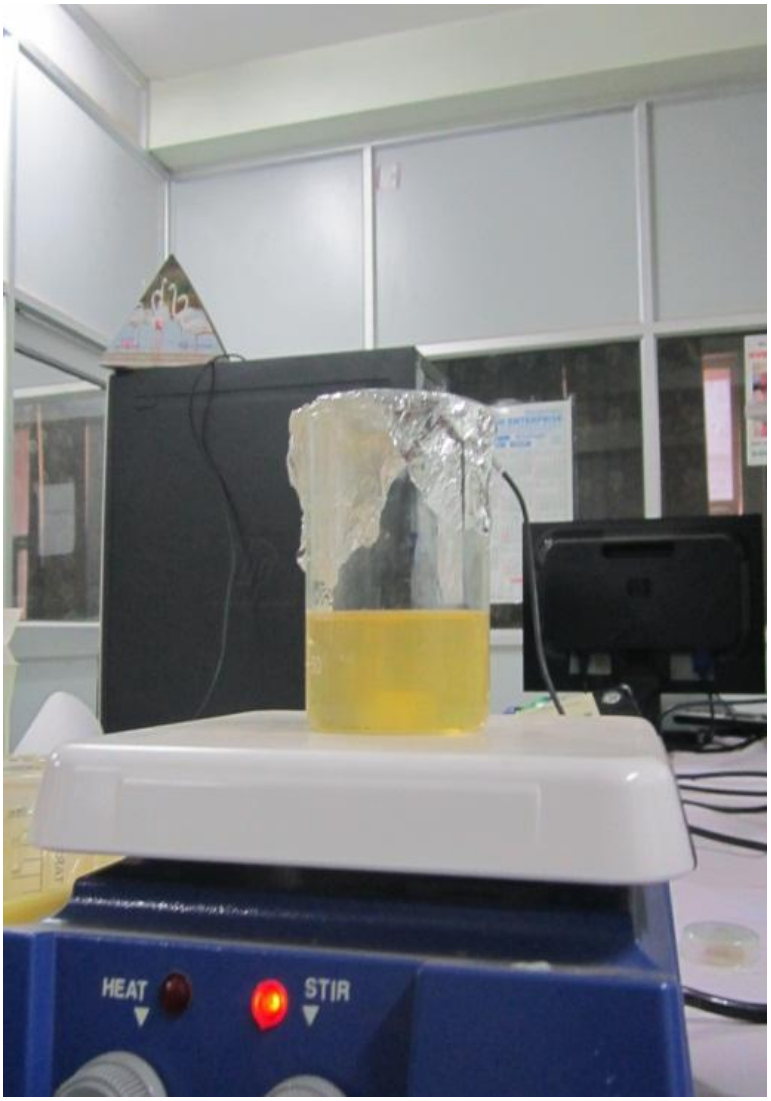


Fig 3.2.8: Chitosan solution on a magnetic stirrer

3.2.3 TPP solution preparation:-

- 10% TPP solution was prepared by adding 5gm of TPP solution in 40 ml of water.
- To reduce its pH to 4 acetic acid was added dropwise till the pH meter shows its pH 4.
- To maintain its concentration to 10% the total volume(acetic acid + water) should be 50ml.
- The below tables shows the volume of added water and acetic acid to maintain both its concentration and pH.

- Chitosan is polycationic and having –NH+ 3 sites when dissolved in acetic acid. In water Sodium tripolyphosphate (Na5P3O10) dissociates to give both phosphoric and hydroxyl ions. When the pH of TPP was adjusted to 4.06, only phosphoric ions will interact with the –NH+ 3 of chitosan.
- Thus, cross-linking was effected by deprotonation at higher pH of TPP, while complex at lower pH the chitosan–TPP was formed by the ionic interaction between positively charged chitosan and negatively charged phosphoric ions.

Water	Acetic acid
40ml	2ml
1ml	2ml
1ml	2ml
1ml	1ml

Total volume= 50ml

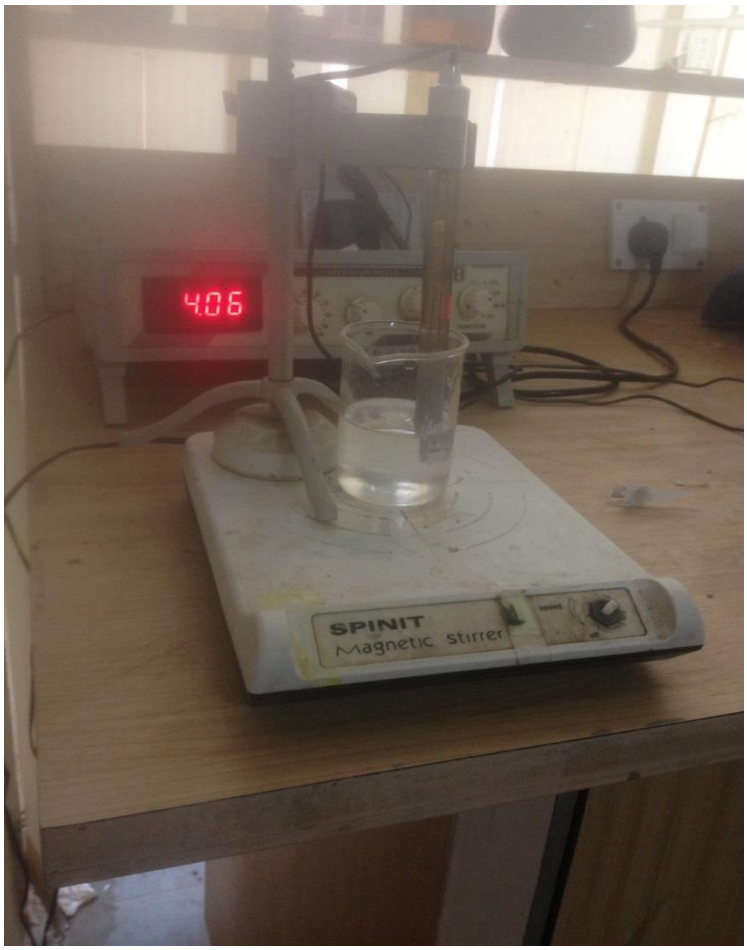


Fig 3.2.9: 10% TPP solution preparation

3.2.4 Chitosan microbeads preparation:-

- 1ml of Chitosan solution was put into syringe with the help of a dropper in such way that there was no air bubbles present in that syringe.
- 5ml of freshly prepared 10%TPP solution was put at bottom of 50 ml centrifuge tubes.
- The syringe containing chitosan solution was inserted into the hole made in the cap of the centrifuge tube and this assembly was tightly fitted with the centrifuge tube. The tip of the needle shouldn't touch the TPP solution.
- Both the Syringe set and centrifuge tubes were put inside the centrifuge chamber. The rpm, time and temperature were set.
- Here we had set its rpm to 500, 700 and 1000 for each concentration.
- Then the machine was run for 5 minutes at room temperature.

Fig 3.2.10: Centrifuge machine

Fig 3.2.11: Rotating unit inside the centrifuge machine

- Under the action of centrifugal force the whole set up inside the centrifuge chamber was came to horizontal which makes the chitosan solution comes out from the tip of needle and comes in contact with TPP cross linker.
- Then crosslinking process starts which ultimately leads to bead formation.
- A surfactant Tween 20 was added to the TPP solution because it slows down the travelling speed of chitosan microbeads across the cross linker there by smoothening its formation.
- The microbeads formed were observed under microscope.

Fig 3.2.12: Microscope

Chapter 4

RESULTS AND DISSCUSSIONS

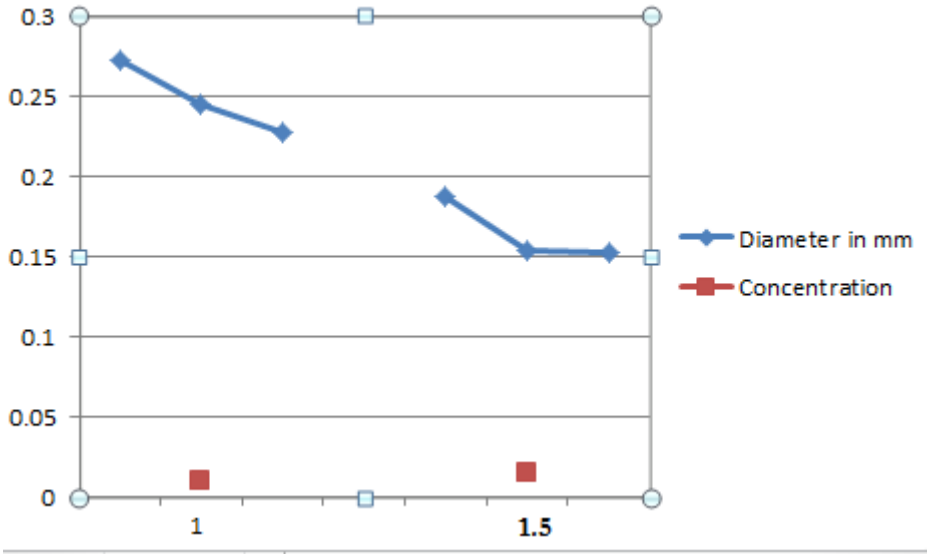
RESULTS AND DISSCUSSIONS:-

Different concentrations (1%, 1.5%) of chitosan solution were taken in 2ml syringe which was then inserted into the centrifugation chamber along with 10% TPP solution. The rotation speed of centrifuge machine was set to 500rpm, 700rpm, and 1000rpm respectively each for different concentration (1%, 1.5%, 2%, 2.5%) of chitosan solution for 5 minutes which resulted in formation of microbeads of different diameters. The table below shows the obtained diameter value of microbeads for different concentration and rpm values.

Microbeads observation:

Chitosan concentration	Run time	500 rpm	700rpm	1000 rpm
1%	5 min.	.272mm	.245mm	.227mm
1.5%	5 min.	.187mm	.154mm	.152mm
2%	5 min.	No microbeads found	No microbeads found	.136mm
2.5%	5 min.	No microbeads found	No microbeads found	.227mm(1200rpm)

A graph was plotted between concentration and diameter.



The microbeads formed were seen under the microscope and the microscopic images were taken.

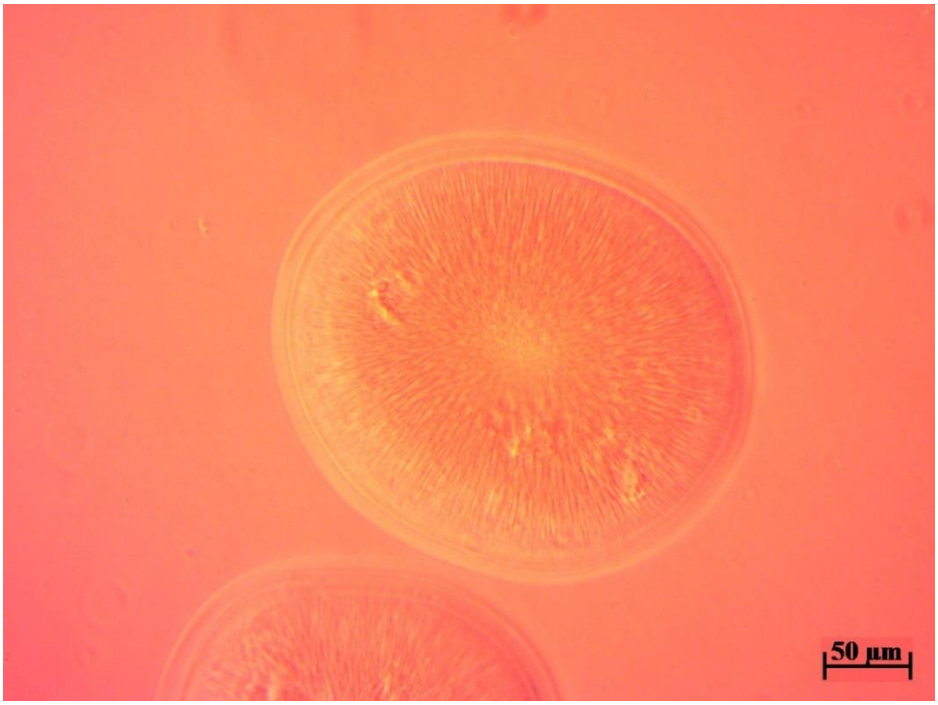


Fig 4.1: 1% chitosan microbeads (500rpm)

Fig 4.2: 1% chitosan microbeads (700rpm)

Fig 4.3: 1% chitosan microbeads (1000rpm)

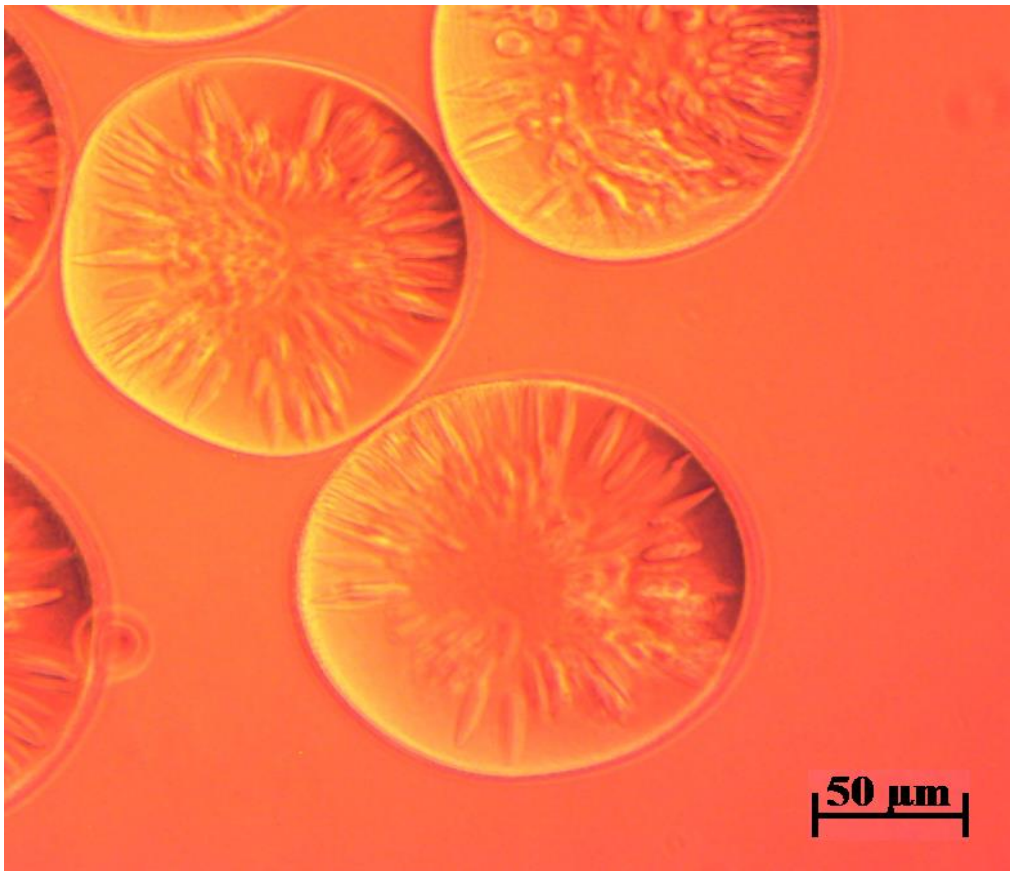


Fig 4.4: 1.5% chitosan microbeads (500rpm)

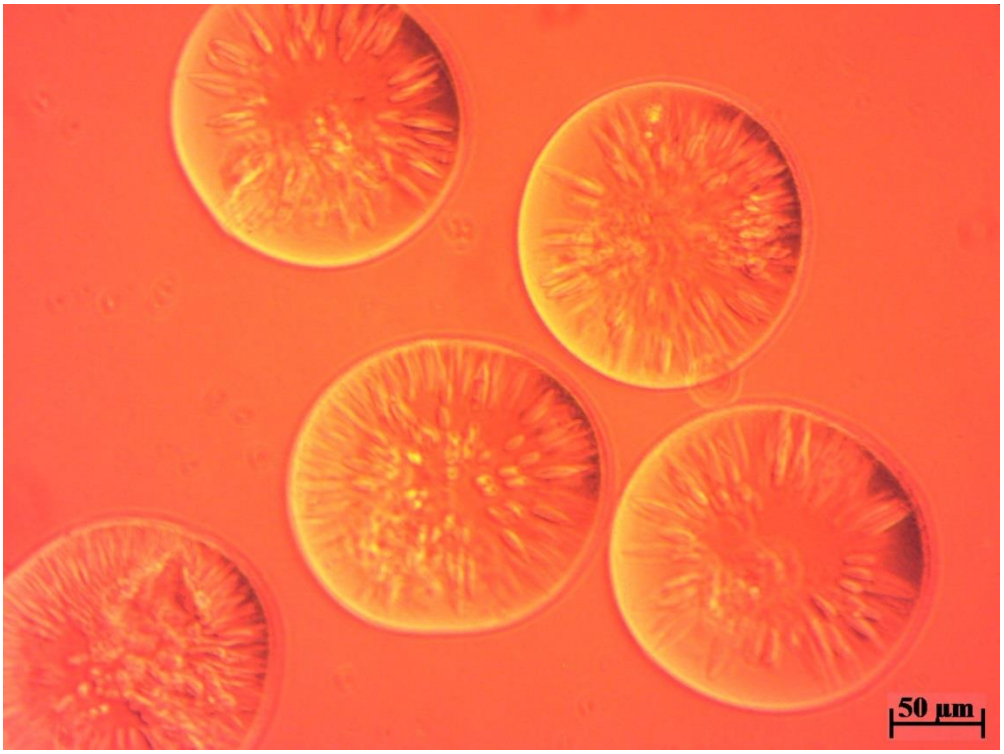


Fig 4.5: 1.5% chitosan microbeads (700rpm)

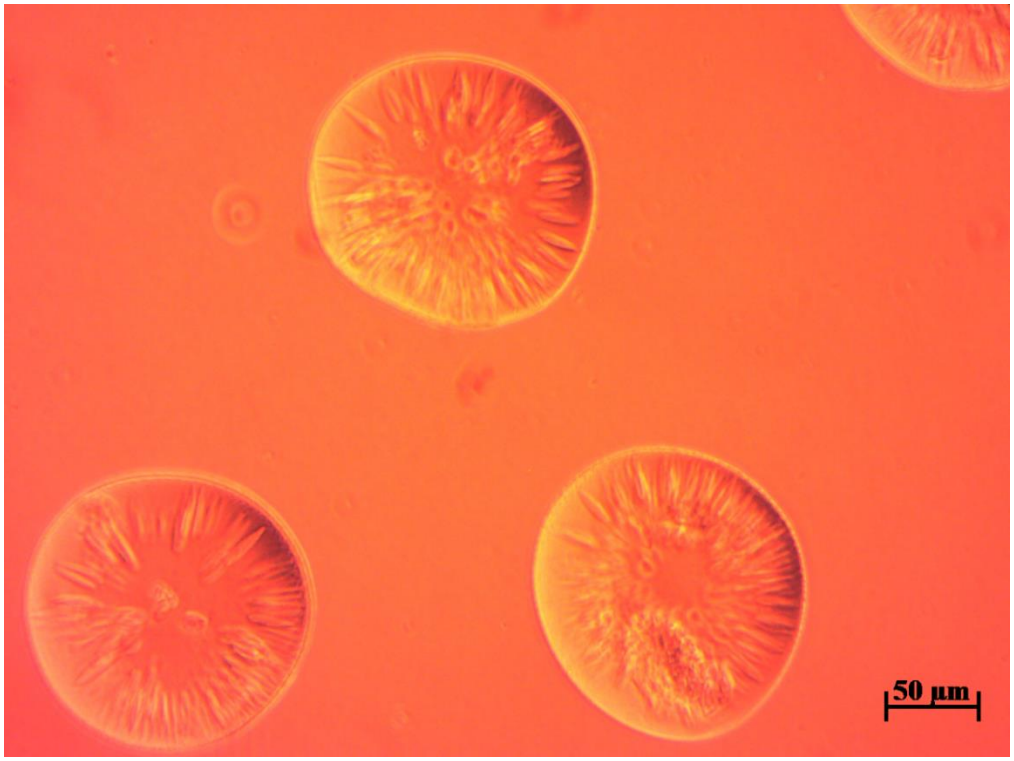


Fig 4.6: 1.5% chitosan microbeads (1000rpm)

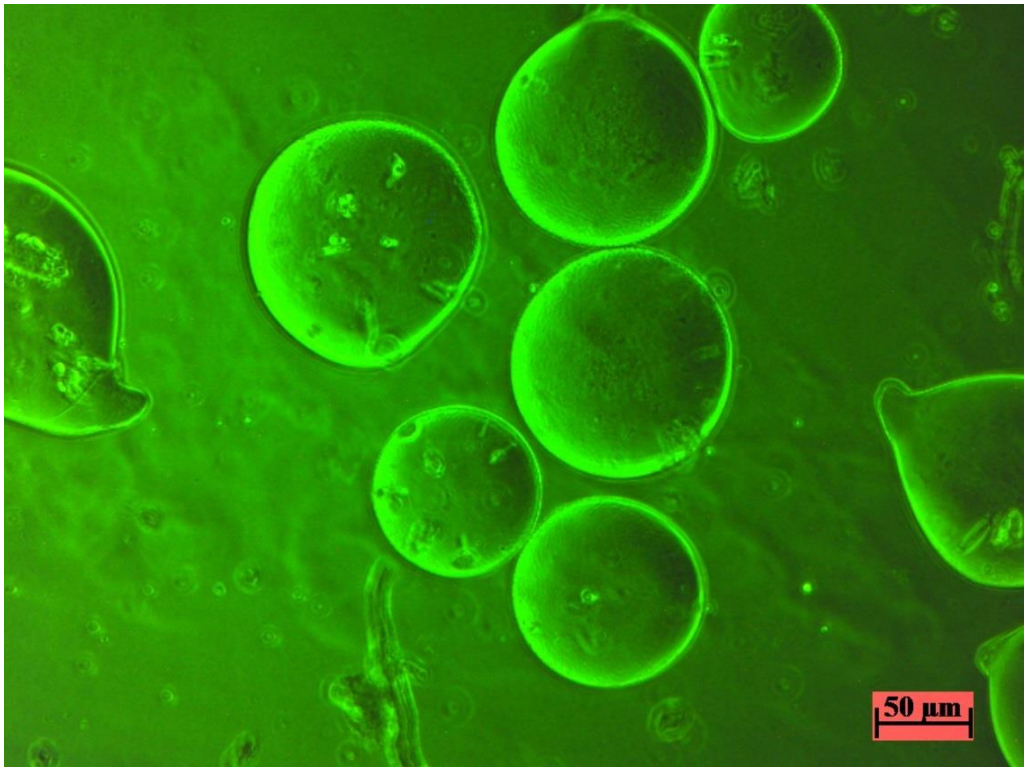


Fig 4.7: 2% chitosan microbeads (1000rpm)

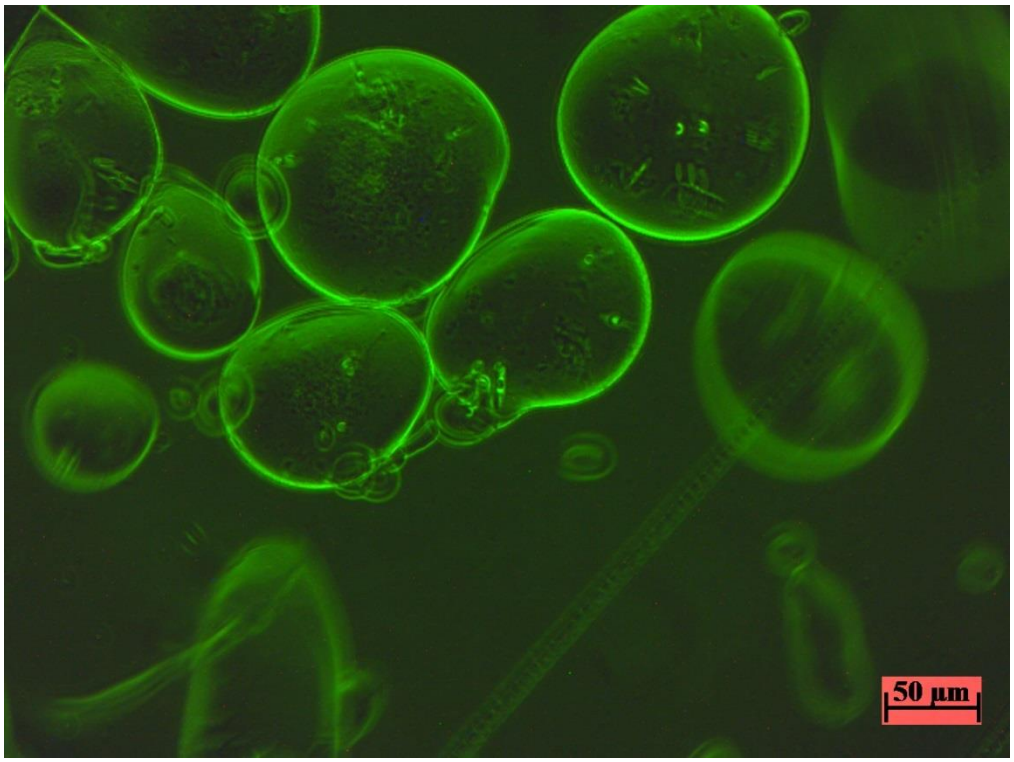


Fig 4.8: 2.5% chitosan microbead (1200rpm)

It can be observed from the above microscopic images as follows:-

- The diameter of the microbeads decreases when rpm was increased from 500 to 1000 at any particular concentration of chitosan solution.
- As the rpm was increased it is found that the microbeads were becoming more uniform and spherical shape and the affect is prominent for 1% and 2% chitosan concentration.
- The factors influencing the diameter of microbeads are :
  - ✓ Viscosity and surface tension of the chitosan solution
  - ✓ Concentration of the TPP solution
  - ✓ Surfactant used
  - ✓ Distance between the tip of the needle to the upper level of the TPP solution present which affects the spherical shape of the chitosan microbeads and tail formation
  - ✓ The volume of the TPP solution which also affects the distance
  - ✓ The centrifugal force(at different RPM) which affects the diameter

Chapter 5

CONCLUSION

CONCLUSION:

Design of a low cost apparatus was made successfully for the cheap and reliable production of chitosan microbeads using centrifugally driven flow. Chitosan microbeads were formed of various concentrations (1%, 1.5%, 2%, and 2.5%) with different rpm (500rpm, 700rpm, and 1000rpm). We got the best result for low concentration values (1%, 1.5%).

Chapter 6

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REFERENCES:

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